AN EXPERIMENTAL STUDY OF POLYDISPERSE BACTERIAL AEROSOLS, REPORT III

A Study of Diphtheria Bacillus Aerosols

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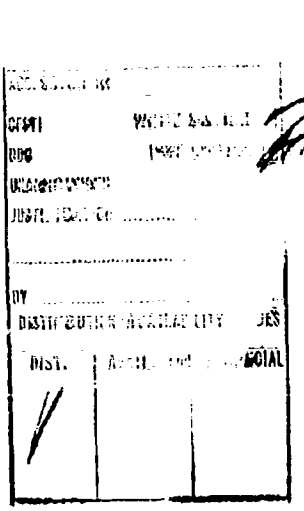

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A Study of Diphtheria Bacillus Aerosols

/Following is the translation of an article by V. P. Zhalko-Titarenko, Kiev Institute of Epidemiology and Microbiology, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 2, 1965, pages 68-73. It was submitted on 1 Jul 1963. The previous reports in this series were published in issue No 10, 1964 and issue No 1, 1965. Translation performed by Sp/7 Charles T. Ostertag Jr./

The method developed by us for the determination of the survival rate of microbes in polydisperse systems makes it possible (reports I and II) to more completely calculate the physical changes in an aerosol during an experiment and therefore avoid misrepresentation in the end result due to sedimentation and coagulation.

In approaching the investigation of the survival rate of the diphtheria bacillus in an aerial medium we undertook the mission to clear up the dynamics of this process in a polydisperse system under various conditions.

We were mainly interested in the fate of the causative agent under conditions which were as close as possible to a natural situation. With this aim we conducted the experiments by spraying a suspension of diphtheria bacilli in saliva in a situation with a room microclimate. In order to clear up what significance saliva has on the survival rate of the diphtheria bacillus in the air, a series of tests were set up with aerosols made up only of cells of washed microbes. This model was formed from an aqueous suspension of washed microorganisms. The rapid evaporation of water left "bare" cells in the air. Such models made it possible to study the direct influence of the aerial medium on the causative agents suspended in it and to determine, in this manner, the role played by the membrane from the saliva. A study was also made of the influence of humidity and temperature on the survival rate of the diphtheria bacillus.

For spraying we prepared a suspension of diphtheria microbes (strain PW-8) which were washed three times. The prepared suspension of cells was relieved of multicellular complexes by means of centrifuging at special rates, after which it was diluted to a concentration of 10 -14 billion microbial cells in 1 ml. The samples for determining the necessary parameters were taken immediately after spraying and also after 10, 45, 60 and 120 minutes.

The results of determining the concentration of live microbes, the concentration of particles and the average content of microbes in a particle of the aerosol were recalculated, for the purpose of determining the survival

rate, according to the formula:

$$b_0 = \frac{C_V}{C_0} = \frac{C_V}{h \cdot C_c}.$$

We took the first determination as 100% and the remainder were calculated depending on this value ("relative survival rate"):

$$B_n = \frac{b_n}{b_1} \cdot 100 \quad \text{or} \quad B_n = i_n \frac{b'_n}{b'_1} \cdot 100,$$

where $b' = \frac{C_V}{C_c}$, $i_n = \frac{h_1}{h_n}$,

b_0 -- degree of survival rate (relationship of live and all the microbial cells in general in the population); B -- relative survival rate; C_V -- concentration of live microbes in the aerosol; C_0 -- overall cellular concentration in the aerosol; C_c -- concentration of particles of the aerosol; h -- average number of microbes in a particle.

The rate of necrosis K was calculated by the known formula:

$$B_n = B_1 \cdot e^{-(kt)}$$

or

$$-K = \frac{1}{t}\left(\lg \frac{B_n}{B_1}\right) \cdot 2.302.$$

For the purpose of obtaining comparable results we followed the example of other authors and calculated K, without multiplying by the modulus 2,302.

For calculating the value of b'_0 we used the following working formula:

$$b'_0 = \frac{S \cdot g \cdot O \cdot \vartheta \cdot W}{M v V \cdot n \cdot a} \cdot 1000,$$

where V -- the volume of the aerosol which passed through the bacteria trap; O -- the volume of liquid in the bacteria trap or the amount of water (in ml) in which the aerofilter was dissolved; g -- multiplicity factor of subsequent dilutions of the preceding amount; v -- volume of liquid, sown in the dish with the nutrient medium; M -- number of sown dishes; S -- total of colonies which grew in all the dishes; ϑ -- effectiveness of the bacteria trap; W -- volume of the aerosol which passed through the VDK ultramicroscope; a -- proportionality coefficient of the membrane with which the reading was made; 1,000 -- common multiple, eliminating the appearance of a fractional result; n -- number of particles counted in the ultramicroscope.

An experimental check of several commonly used media showed that for the most complete exposure of live diphtheria microbes from the air media containing blood are necessary, 5% blood agar, Klauberg's medium, chocolate-tellurite agar,

Pyushel's medium. On serum or common meat-peptone agar diphtheria bacilli are hardly seeded out of the air. On the basis of this, in our investigations we used only the 5% blood agar.

The aerosols of diphtheria bacilli in saliva were obtained from an aqueous suspension of washed microbes and settled, native, human saliva which were mixed in equal volumes. The microclimate in the chamber was as follows: Temperature 18--20°, humidity 60 $\pm$ 1%. The data presented in figure 1 makes it possible to distinguish three phases in the process of necrosis. The first phase lasted around 45 minutes and the survival rate hardly changed ($K = 0$). During the next 15 minutes the survival rate dropped sharply ($K = 32 \cdot 10^{-3}$). Between 60 and 120 minutes this process slowed down ($K = 3.95 \cdot 10^{-3}$). Thus the necrosis of the microbes took place in the last two phases.

In these tests the particles consisted of saliva and bacterial cells. A study of the morphology of such particles (Report II) showed that the water evaporated slowly from them. Dried particles could be detected no earlier than in 45--60 minutes after the onset of the test.

Apparently the graded nature of the curve for the survival rate can be explained in the following manner. In the course of the first few dozens of minutes the particles of the aerosystem contain a sufficient amount of moisture, which protects the microorganisms from desiccation and death. Besides this, primarily during this period there is a predominance in the air of large particles, that is, the conditions for the existence of the microorganism were most favorable. At the end of the period two basic processes converged which contributed to the sharp drop in the curve of the survival rate: The aerosol was relieved of large particles and in the remainder intensive evaporation began. This developed directly on the cells. Thus the role of the substances with the saliva which covered the cell most rapidly of all led to the temporary "self removal" from the process of desiccation which is dangerous for the cell. Apparently as soon as the reserves of water in the covering materials were exhausted, evaporation began directly in the cells of the microorganisms and this exerted a lethal effect on them.

In addition to what was pointed out, tests with microbial particles without covering substances ("bare") also testified in favor of the stated hypothesis. For this, washed diphtheria microbes suspended in distilled water were sprayed in the air. Following spraying the water surrounding the microbe evaporated almost instantly, leaving the pure bacterial cells in the air. Figure 2 presents the results of spraying diphtheria bacilli in water at a temperature of 18--19° and a humidity reaching 60%. The diphtheria bacillus began to die immediately following spraying and the rate of this process remained relatively high ($5.7 \cdot 10^{-3}$) throughout the entire test, approaching the speed of necrosis which was observed in the last phase of the tests with saliva. In this manner it was sufficient to remove from the particles the membrane made from the mucous-protein substances of the saliva and necrosis began to be detected immediately after the formation of the aerosol. This impels one to think that the delay in evaporation, observed in the tests with saliva, and the delay in necrosis are connected together.

If desiccation is capable of exerting an influence on the survival rate then it is appropriate to assume the humidity of the air also affects the diphtheria bacillus. We studied this problem on models with "bare" microbial particles (in order to exclude the concealing influence of the envelope substances) at 18--20° and at various humidities -- from 40 up to 90% (see figure 2). However, the expected changes in moisture were not detected. This result may be explained, first of all, by the fact that the changes were beyond the limits of sensitivity of the method and consequently were not sufficiently great. Secondly, it is possible that this speaks for the natural resistance of the diphtheria bacillus to desiccation, thus smoothing out any possible changes. Finally, the result obtained may have depended on the heterogeneous nature of a polydisperse aerosol, in which the large particles which were predominant in the beginning, being less sensitive to changes of humidity, dominated when the results were calculated.

It is generally known that the survival rate of microorganisms under unfavorable conditions depends also on the temperature of the environment. However, it was not known how and to what degree this property would be manifested in polydisperse aerosols of the diphtheria bacillus. Therefore we studied the survival rate of the diphtheria causative agent in an aerosol at -6° and at 35° (data on the survival rate at an average temperature were obtained in the previous series of tests). The data shown in figure 3 indicates that the humidity in these investigations remained within the limits of average values - 60--61%. The survival rate at 35° turned out to be almost 5 times lower than at an average temperature - 18--20°, and at minus temperatures necrosis was hardly observed.

The study of the survival rate of diphtheria microbes in a polydisperse aerosol was conducted on the basis of a method which excluded the possibility of a mixture of a biological process -- necrosis, and a physical phenomenon -- the sedimentation of particles. However the resulting values of the survival rate were found in a specific dependency on the nature of the polydisperse aerosystem. In the process of sedimentation the specific proportion of large particles in the total mass of the aerosol gradually decreases, consequently the very nature of the system is changed. Strictly speaking, at each given moment of existence of a polydisperse system the investigator is dealing with a changed nature of the aerosol. The general tendency of these changes consists of a decrease in the average particle size and the degree of polydispersity. Thus, the older the aerosol then the more stable it is in relation to the conditions of existence for microorganisms. And the "younger" a polydisperse system is the more predominant are the large particles with a high content of microbial cells in determining the survival rate index. This circumstance makes it possible, with a high degree of probability, to consider that the conditions of existence in large particles are more favorable than in small, since the highest survival rate corresponds to the period of prevalence of large particles.

Thus the quite widespread opinion concerning the supposedly quite favorable conditions for the survival of causative agents which are created in particles following the drying up of the saliva, is untrue, because a prior analysis and experimental verification testify that primarily in large particles

which are rich in moisture the conditions for survival are the best and the level of the survival rate is the highest. It is apparent that in an epidemiological situation these large particles play a more important role.

The survival rate in a polydisperse system, being an overall index, should apparently be different in aerosol particles which are different in size. It is also probable that to some degree the difference in the survival rate indices in polydisperse systems may characterize their nature. Subsequent experimental investigations should show to what degree these proposals are correct.

The study of the survival rate of the diphtheria bacillus in the air made it possible to establish (applicable to the selected model of a polydisperse aerosol) the dynamics of necrosis of the population, whereas the extreme survival rate of this microbe was studied around 30 years ago by Wells. The scientific necessity in the development of methods for investigating polydisperse systems and determining the survival rate of microbes in them we examined in the necessity of studying the process of transmission of the so-called droplet infections (which now would be more correctly named aerosol infections). An interpretation of the nuances in the mechanism of transmission of aerial infections from a sick person to a healthy one would open up, it seems to us, the perspectives for developing principally new prophylactic agents and methods. If it is taken into consideration that the immunoprophylaxis of certain aerial infections, which leave behind an unstable immunity, is very problematic, then the search for other method of combatting them should be considered an unconditionally urgent task.

Conclusions

1. As a result of a study of the survival rate of diphtheria microbes in an aerosol (beginning with the 3rd minute following spraying and continuing for the next 120 minutes) it was established that it is found in dependence on the evaporation of water: In drops with slowed down evaporation (saliva) the survival rate turned out to be higher than in the rapidly evaporating aqueous particles.

2. The necrosis of microbes in an aerosol increased sharply at a temperature of 35° and at a temperature below zero the viability of all the diphtheria cells was preserved.

3. The highest survival rate for diphtheria microbes was noted in the aerosol during the period when there was a prevalence of large particles. In the presence of small droplet fractions their rate of necrosis increased.

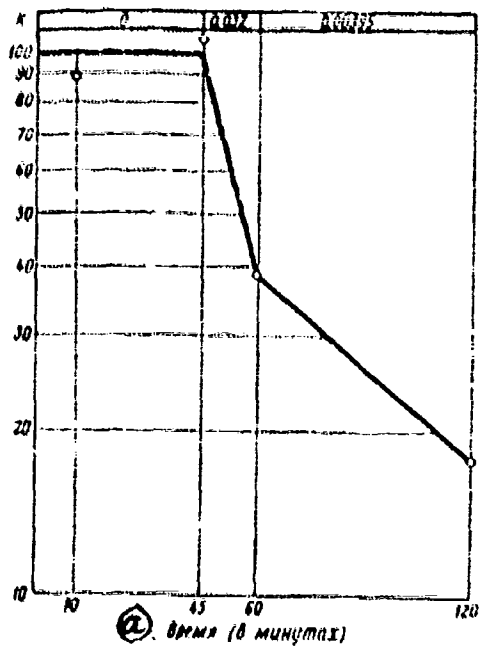

Figure 1. Survival rate curve for diphtheria bacilli in an aerosol with saliva. a - time (in minutes)

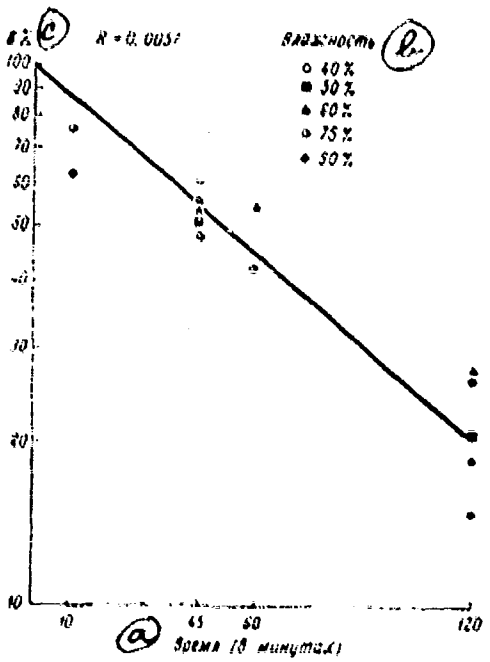

Figure 2. Survival rate curve for diphtheria bacilli in an aerosol on an aqueous base. a - time (in minutes); b - humidity; c - in %.

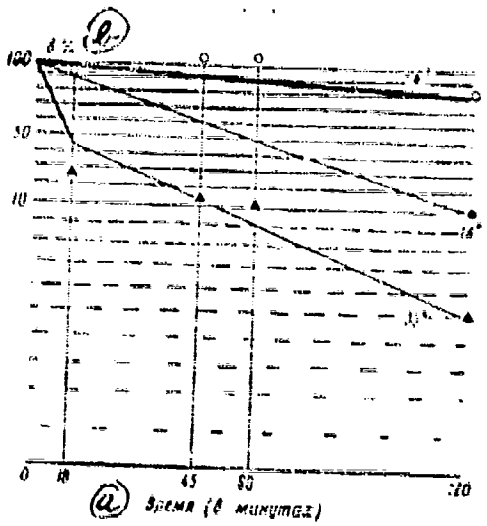

Figure 3. Survival rate curves for diphtheria bacilli in an aerosol at various temperatures. a - time (in minutes); b - in %.